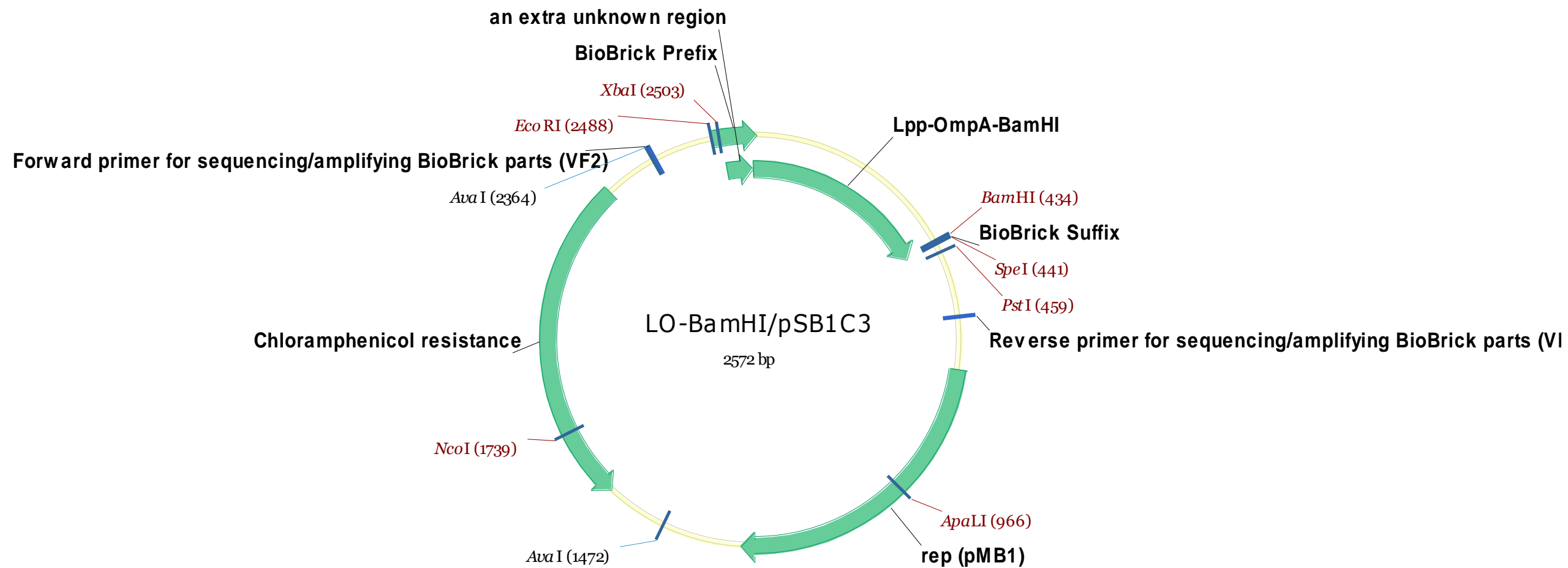


LO-BamHI/pSB1C3



Strategy

- Vector: pSB1C3 (2070 bp) → EcoRI + SpeI
- Insert: EcoRI-XbaI-Lpp-OmpA-BamHI-SpeI amplified by PCR → EcoRI + SpeI
 - Template: NCTU #3_BBak_K1694035_pSB1C3 (#3) Pcons-RBS-Lpp-OmpA-NcoI/pSB1C3
 - PCR product size: 468 bp

Primer Design

- Lpp-XbaI-EcoRI-F:

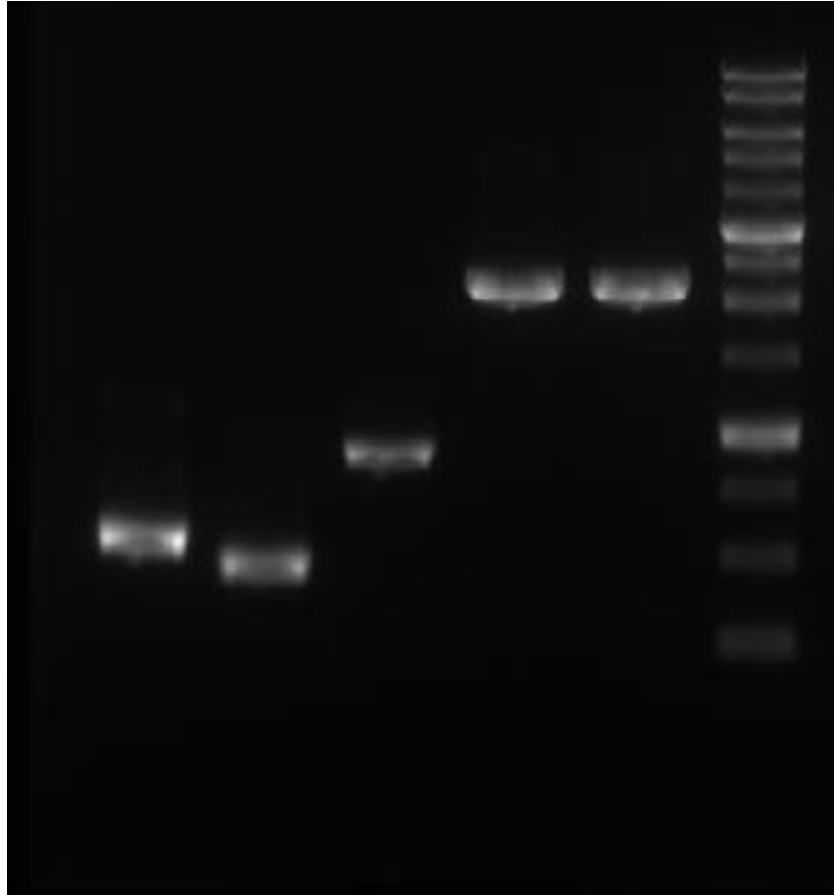
- 5'-

- AGTCAAGAATTCGCGGCCGCTTCTAGATGAAAGCAACCAAGCTG
 - 3'

- OmpA-BamHI-SpeI-R:

- 5'- AAAGAGACTAGTAGGATCCACGTGTGGCAATTTCGGGGTGA
 - 3'

PCR with KOD-plus



- Date: 2016.08.17

- Lane:

1. Pcons-RBS-LO-BamHI

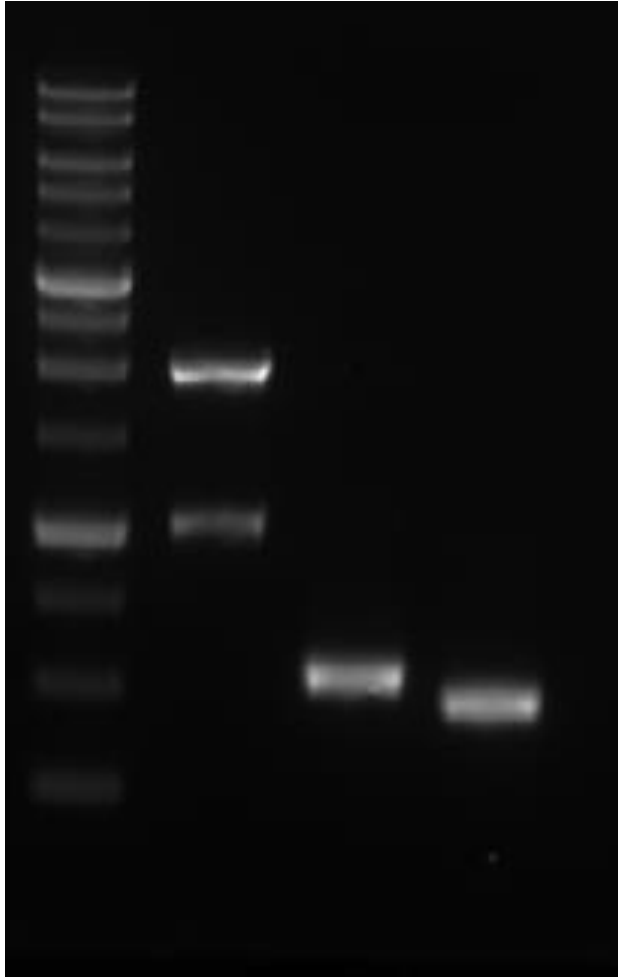
2. LO-BamHI

3. GFP

4. AOX1

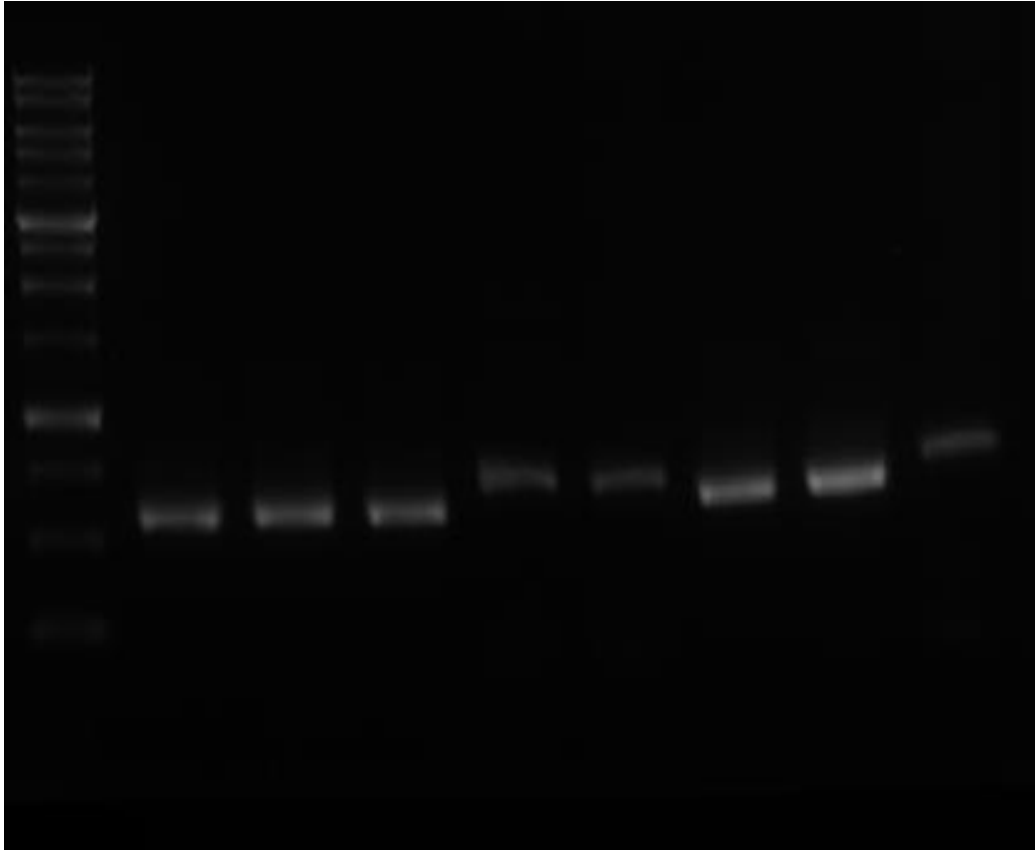
5. AOX2

Ligation



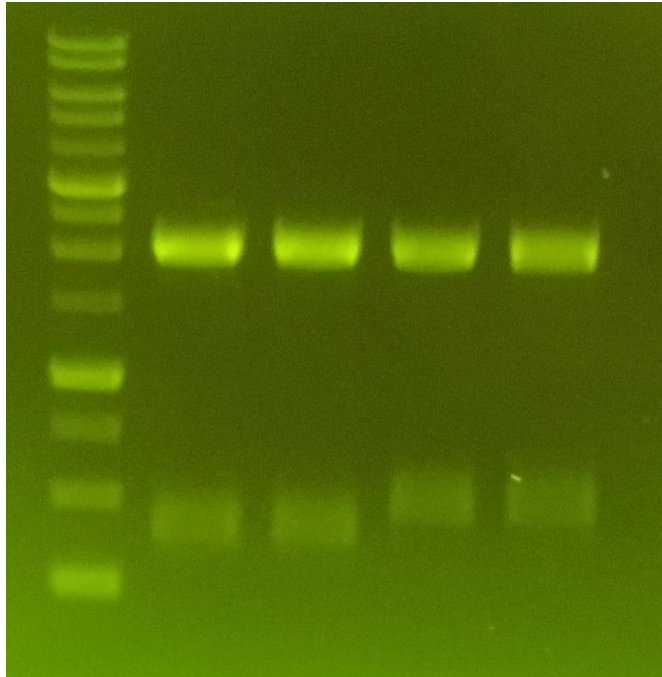
- Date: 2016.08.18
- Lane:
 1. pSB1C3: EcoRI + SpeI
 2. Pcons-RBS-LO-BamHI: EcoRI + SpeI
 3. LO-BamHI: EcoRI + SpeI

Colony PCR



- Date: 2016.08.19
- Clone: LO-BamHI/pSB1C3
- Primer:
 1. VF2
 2. LO-BamHI-SpeI-R
- Lane: #L1 ~ #L8
- #L2, 3 → Plasmid extraction
→ RE check

RE check



- Date: 2016.08.23
- Clone: LO-BamHI/pSB1C3
- RE: EcoRI + PstI
- Lane:
 1. #L2
 2. #L3
 3. #P6 (Pcons-RBS-LO-BamHI/pSB1C3)
 4. #P7 (Pcons-RBS-LO-BamHI/pSB1C3)
- #L2 → Sequencing

Sequencing

- Date: 2016.08.27
- Sequencing primers: VF2
- Clone: LO-BamHI/pSB1C3 #L2
- File: “2016.08.27_Pcons, LO-BamHI_pSB1C3(#P6, #L2)”
- Result: **correct** → make a stock